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Cancer Progression

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14. ABSTRACT The purpose of this Idea Development Award is to understand the molecular basis of early events in prostate cancer progression. In particular the proposal focuses on a class of non-coding RNAs called microRNAs that function to suppress large networks of genes during cell fate transitions. The proposal was based on preliminary data showing that in absence of all microRNAs, prostate tumors associated with PTEN loss fail to progress. The goal here is to determine the microRNAs and downstream-regulated pathways responsible for this striking block. In the past year, we have crossed in reporters into our models that allow us to isolate the transformed epithelial cells from the otherwise highly heterogeneous cellular context of the prostate. RNA has been produced from these cells and are being profiled for the microRNAs and mRNAs, which will then be evaluated computationally to uncover networks of microRNA-mRNA interactions that normally drive progression. The significance of this work is that it will provide both markers of early progression as well as potential nodes within the networks that can be manipulated diminishing the requirement of more aggressive treatments.					
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Introduction:

The overall goal of this funded proposal is to dissect the molecular mechanisms of early tumor progression in the prostate. The proposed aims are based on preliminary findings showing that the loss of a specific class of regulatory molecules called microRNAs blocks progression from early hyperplasia to dysplasia in a PTEN knockout model of the disease. The aims themselves are to uncover the underlying microRNAs (aim 1) and microRNA regulated pathways (aim 2) that are normally responsible for progression. Knowing the miRNAs and downstream pathways that cooperate with PTEN loss (the most commonly deleted gene in prostate cancer) has the potential to improve the evaluation of the risk of early progression. The ability to distinguish and treat tumors that are likely to progress will have a broad impact on the disease as it would have the potential to diminish the overdiagnosis and overtreatment of early disease.

Body:

The goals for year one as outlined in the statement of work were to:

- 1) Cross our Pten, Dgcr8 double knockout model to a YFP reporter to allow isolation of the effected cells from the otherwise heterogeneous prostate (months 1-9)
- 2) Age desired genotypes for approximately 3 months (months 6-9)
- 3) Isolate prostates from the aged mice, dissociate into cells, and isolate YFP positive cells using flow cytometry (months 6-9)
- 4) Prepare RNA from samples preserving both long and short RNA fractions (month 10)
- 5) Profile RNA samples for miRNA and messenger RNA species (months 11-13)

Our progress in each of these areas is as follows:

- 1) We have successfully crossed in the reporter into the mutant mice producing the following number of mice of each genotype:
 - a. Probasin-cre, Pten flox/flox, Dgcr8 flox/flox, Rosa26-lox-stop-lox-YFP (n=11)
 - b. Probasin-cre, Pten flox/flox, Rosa26-lox-stop-lox-YFP (n=15)
 - c. Probasin-cre, Dgcr8 flox/flox, Rosa26-lox-stop-lox-YFP (n=15)
 - d. Probasin-cre, Rosa26-lox-stop-lox-YFP (n=20)
- 2) Male mice of above genotypes were aged to between 11 and 14 weeks.
- 3) Prostates were isolated (representative image, figure 1). Half of each prostate was processed for histology and immunohistochemistry (representative image, figure 1) and the other half was dissociated and YFP expressing cells were isolated by flow cytometry (representative image, figure 1).

- 4) RNA has been purified from most of the samples and quality of RNA has been confirmed by on a Agilent bioanalyzer (representative bioanalyzer run shown in figure 1). Amount of RNA isolated from each sample ranged between 10-120ng.
- 5) In our application, we proposed to do Affymetrix array profiling for messenger RNAs and multiplex quantitative reverse transcription polymerase chain reaction profiling of small RNAs. Unfortunately, due to technical error in labeling of RNA for arrays, we lost RNA samples from 4 prostates of each genotype. Fortunately, we have enough remaining samples to complete this objective. However, due to advancements in techniques, we are currently researching the possibility of performing RNA sequencing for both mRNAs and small RNAs. We have successfully produced small RNA libraries from as little as 10ng RNA and, therefore, expect success there. We are currently pricing out costs for RNA sequencing versus Affymetrix arrays for mRNA profiling. We expect profiling will be completed by end of month 13 as outlined in our statement of work.

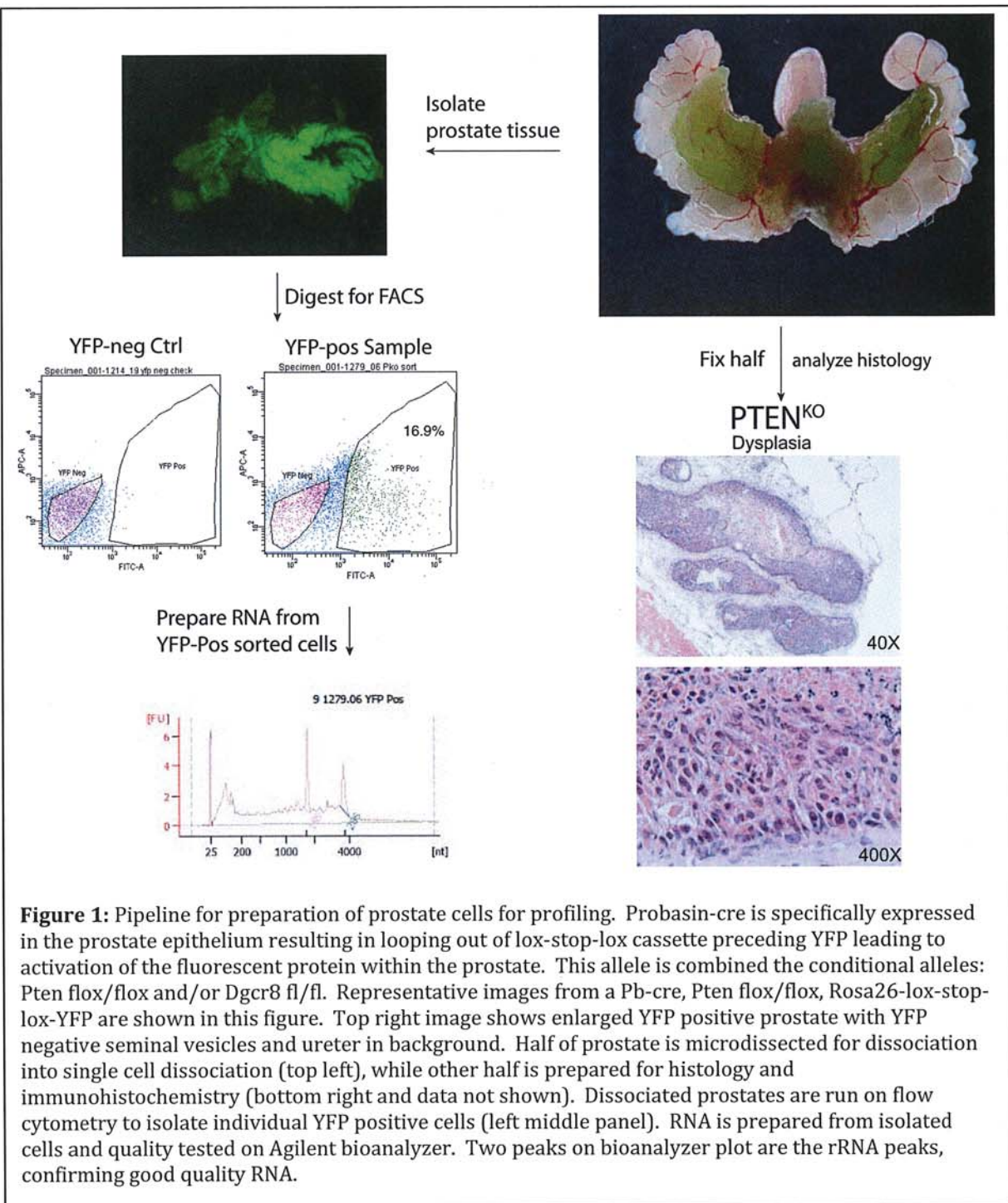


Figure 1: Pipeline for preparation of prostate cells for profiling. Probasin-cre is specifically expressed in the prostate epithelium resulting in looping out of lox-stop-lox cassette preceding YFP leading to activation of the fluorescent protein within the prostate. This allele is combined the conditional alleles: Pten flox/flox and/or Dgcr8 fl/fl. Representative images from a Pb-cre, Pten flox/flox, Rosa26-lox-stop-lox-YFP are shown in this figure. Top right image shows enlarged YFP positive prostate with YFP negative seminal vesicles and ureter in background. Half of prostate is microdissected for dissociation into single cell dissociation (top left), while other half is prepared for histology and immunohistochemistry (bottom right and data not shown). Dissociated prostates are run on flow cytometry to isolate individual YFP positive cells (left middle panel). RNA is prepared from isolated cells and quality tested on Agilent bioanalyzer. Two peaks on bioanalyzer plot are the rRNA peaks, confirming good quality RNA.

Key Research Accomplishments

- 1) Development of mouse model that allows dissection of microRNA function during early stages of tumor development.
- 2) Development of methods to isolate and evaluate transformed cells from the otherwise heterogeneous cellular background of the prostate.
- 3) Preparation of high quality RNA from small numbers of cells allowing for future measurements of changes in mRNA and small RNA levels between different mutant backgrounds.

Reportable Outcomes

Project is in its early phase. We expect to submit our first manuscript this year describing the phenotype associated with microRNA loss in the Pten null prostate along with profiling of mRNAs and miRNAs. At that time, we will make all profiling data publicly available. Finally, we have used the mouse models we developed for this proposal as preliminary data in a NIH/NCI grant U19 grant on extracellular RNAs, which we were very recently awarded.

Conclusion

In the first year of this Idea Development Award, we have made significant progress on the overall objectives of the proposal and have succeeded at achieving all proposed milestones. We have built the reagents required to tackle central of question of how microRNAs regulate early stages of prostate cancer progression. Answering this question should enable better prognosis and treatment of early stage disease.